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The Biological Activity of *Alhagi graecorum* and *Matricaria chamomilla* Extract Against Some bacteria causing urinary tract infections

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ABSTRACT

Among the most significant bacteria that cause urinary tract infections were *Staphylococcus aureus, Escherichia coli, Psedomonas aeruginosa and Streptococcus pyogenes*, all of which are resistant to numerous antibiotics. The continuous hunt for new sources of antimicrobials has been sparked by the rise of microbes resistant to common antibiotics.

The leaves and roots of *Alhagi graecorum* and *Matricaria chamomilla* were extracted. Each plant extract's antibacterial activity is assessed using the disk diffusion method.are used for detection Minimum inhibition concentration and bactericidal concentration. The ethanol extract of *Matricaria chamomilla* extract was the most effective against *S. aureus* and *P. aeroginosa*, while *Alhagi graecorum* extract was the most effective against *E. coli*, and *S. pyogen*, therefore a viable choice for defense against harmful microbes and can be utilized in the synthesis of antibacterial medicines.

In broth microdilution method, the extract of chamomile leaves showed inhibitory effect and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined as 13.88 and 15 mg/mL, respectively.

Keywords:*; Alhagi graecorum,* Biological Activity, *Matricaria chamomilla*, Minimal inhibition concentration, Minimal Bactericidal Concentration, Plant Extract.

1. Introduction

In addition to saving many lives, the development of antibiotics in the 20th century made it possible for risky medical procedures including organ transplantation, open heart surgery, and cancer therapies

[1]. However, drug-resistant bacteria have emerged as a result of antibiotic overuse and misuse [2]. These have a major social and economic impact and result in millions of deaths or disabilities every year. The World Bank estimates that by 2050, multidrug-resistant bacteria may cause an annual loss of "$3.44 trillion" [3] in global GDP and up to "$1 trillion" in additional healthcare costs [4].

According to "World Health Organization (WHO)" statistics, humans' careless use of conventional antibiotics in agriculture, animal husbandry, the food industry, and healthcare is killing about 10 million people globally [5]. The most prevalent infection in humans, upper respiratory tract infections (URTIs), are mostly brought on by bacteria and viruses. Numerous disorders are caused by *Streptococcus pyogenes*, a significant bacterial pathogen of the upper respiratory tract [6]. It is the most frequent cause of bacterial pharyngitis and is associated with a number of dangerous side effects. However, it appears that URTIs are frequently treated with needless and illogical self-medication with antibiotics [7], which leads to resistance to numerous bacteria, including *S. pyogenes* [8].
Food poisoning, pleuropulmonary disease, medical device-related bloodstream infections, skin and soft tissue diseases and even infective endocarditis or osteomyelitis can all be brought on by *Staphylococcus aureus* [9]. Another significant *Staphylococcal* pathogen is *Staphylococcus warneri*, which is typically linked to hospital infections such sepsis, meningitis, endocarditis, skin and soft tissue diseases, and infections mediated by catheters or implanted devices [10]. Numerous bacterial illnesses in both humans and animals are linked to *Streptococcus* species. Human illnesses include arthritis, meningitis, pneumonia, and newborn sepsis, but in animals they primarily cause mastitis [11]. Due to bacterial evolution and antibiotic abuse *S. aureus* drug resistance has steadily increased. The rising incidence of methicillin-resistant Staphylococcus aureus (MRSA) infections globally has made clinical anti-infective treatment of MRSA more challenging [12].

E. coli and Klebsiella pneumonia were the most often isolated aerobic Gram-negative bacteria from early infections. After surgery, *Pseudomonas aeruginosa* was the most frequent infection. After that, *K. pneumoniae, E. coli, and Enterobacter* are most commonly isolated. The new threat is the severe issue of widespread vancomycin resistance. Antibiotic-resistant bacteria have traditionally been handled with a bias for Gram-positive microorganisms since vancomycin has caused public health concerns [13]. Throughout this study, the more broad term pharyngitis refers to acute inflammation of the tonsils, pharynx, or both. The most common symptom of pharyngitis is throat pain. Although

viruses are the most prevalent cause of pharyngitis", the most common bacterium detected during acute pharyngitis is *S. pyogenes*, often known as group A β-haemolytic Streptococcus (GAS). GAS pharyngitis is projected to affect 450 million children worldwide each year [14]. Chronic respiratory system disorders are caused by the versatile pathogenic agent *Pseudomonas aeruginosa*. in individuals with cystic fibrosis and nosocomial infections in immunocompromised hosts. This type of bacterium causes a wide range of systemic ailments, including dermatitis, urinary tract diseases, soft diseases, bacteremia, bone and joint diseases, gastrointestinal diseases, and respiratory system problems [15]. Numerous experts from around the world have looked into the antibacterial properties of various medicinal plants. Iraq has a wide variety of medicinal plants. Among these are the well-known medicinal flowering plant *Matricaria chamomilla* (German chamomile), which is a member of the Asteraceae family and grows in temperate regions of Europe, Asia, America, and Africa. It has a wide range of effects, including antimicrobial and antioxidant properties [12]. Since *S. pyogenes* was isolated from upper respiratory tract illnesses, the purpose of this investigation was to assess the antibacterial activity of a few medicinal plants that are frequently used in traditional medicine for bacterial infections [16].

The majority of UTIs are linked to bacterial contamination, specifically gram-positive bacteria like *Streptococcus pyogenes* and *Staphylococcus aureus* and gram-negative bacteria like *E. coli*. It is crucial to conduct research on the effectiveness of the following plant extracts: German chamomile *Matricaria chamomilla* and *Alhagi graecorum*. In order to assess the antibacterial activity of these plant extracts against urinary tract infections brought on by *Psedomonas aeruginosa, Streptococcus pyogenes, E. coli, and Staphylococcus aureus* in vitro, this study was conducted.

2. Materials and Methods

2.1 Preparation of Plant Extract

Two plant species were gathered from the Iraqi local market, then disinfected, and allowed to dry in the shade. Each dried plant parts were ground into a fine powder and sieved through a 100 mm screen. After soaking 50 g of the fine powder was dissolved in 200 ml of ethanol for 48 hours with stirring, then filtered through two layers of gauze, centrifuged for 8 minutes at 10,000 rpm, and filtered through Whatman filter paper No.0.6

A rotary vacuum evaporator was used to evaporate and dry the goods at 40 °C with lowered

pressure. The extracts' yield ratios were computed using the following formula after they were weighed and kept in little bottles at 5 °C in a refrigerator: R/S = 100" is the yield ratio of extracts, where R is the weight of the plant residue that was extracted and is the weight of the original plant sample.

2 .2 Isolation and Identification of Bacteria

Four bacterial strains that cause urinary tract infections were used to test antibacterial activity of plant extract's. Gram-positive bacteria *Streptococcus pyogenes and Staphylococcus aureus* Gram-negative *Escherichia coli and Pseudomonas aeruginosa*. The bacterial strains were from the University of Baghdad, department of botany and Microbiology.

2.3 Preparation of Bacterial inoculum

Mueller-Hilton agar slants were used to culture each bacterial strain for 24 h. at 37 ℃. A spectrophotometer was used to harvest the bacterial growth using 5 ml of sterile saline water, adjust its absorbance at 580 um, and dilute it until the viable cell count reached 10^7 CFU/ml [17].

2.4 Detection of Minimum inhibitory concentration

Each plant extract's antibacterial activity is assessed using the disk diffusion method. To achieve a final concentration of 10 mg/disc, the 50 mg of plant extract leftovers were redissolved in 2.5 ml of ethanol, sterilized using a Millipore filter (0.22 mm), and then put onto sterile filter paper discs (8 mm in diameter). In order to achieve 10^5 CFU/ml of media, 10 ml of Mueller-Hilton agar medium (as a basal layer) and 15 ml of seeded medium that had already been infected with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) were added to sterile Petri plates.

Mueller-Hilton agar plates were covered with sterile filter paper discs supplied with a plant extract concentration of 10 mg/ml. As a positive control, filter paper discs containing 5 mg of Gentamycin were utilized. To allow the plant extracts to diffuse, the plates were refrigerated at 5 °C for two hours, and then they were incubated at 37 °C for twenty-four hours. Using a Vernier caliper, the presence of inhibitory zones was observed, noted, and regarded as a sign of antibacterial activity [17].

The lowest concentration of an antimicrobial agent that stops microbial growth during a 24-hour incubation period is known as the minimum inhibitory concentration, or MIC. The disk diffusion method was used to alter the MIC for the most effective plant extracts, which showed strong antibacterial activity at 10 mg/ml. Their effectiveness against bacterial strains that cause UTIs was

then assessed. 75 mg of active plant extracts were dissolved in 2.5 ml of ethanol, sterilized with a Millipore filter, and then the necessary amount was placed on sterile filter paper discs (8 mm in diameter) to create different concentrations of the extracts (2.5, 3.5, 7.0, 12.0, 15.5, and 18.0 mg/m1).

Sterile Petri dishes were filled with Mueller-Hilton agar, and bacterial suspensions of pathogenic strains were filtered. On the Mueller-Hilton agar plate surface, filter paper discs containing varying amounts of the active plant extract were positioned. After being refrigerated at 5 °C for two hours, the plates were incubated for twenty-four hours at 37 °C. Using a Vernier caliper, zones of inhibition were measured and noted in relation to the amounts of the active plant extracts.

2.5 Detection of Minimum Bactericidal Concentrations (MBCs)

Streaks were transferred onto sterile Tryptone soy agar (TSA) plates from the two lowest concentrations of the plant extract plates that showed invisible growth (from the inhibition zone of MIC plates) and subcultures. After 24 hours of incubation at 35 °C, the plates were checked for bacterial growth in relation to the content of plant extract. According to CSLI, 2018 [18], MBC was defined as the concentration of plant extract that did not show any bacterial growth on the newly infected agar plates.

2.6 Phosphate Buffer Saline Extraction of Antibacterial Peptides

Using a mortar and pestle that had been refrigerated and 4.5 milliliters of phosphate buffer saline (PBS), the most recent 0.3 grams of roots and flowers from each sample were ground into a powder. After that, the specimens underwent three freeze-thaw cycles with a 12-hour interval between each cycle. After that, tubes were centrifuged for about ten minutes at 10,000 rpm. For quantification, the resultant supernatant was gathered and stored at 4 °C.

2.7 Measurement of Protein Concentration

spectrophotometry, the total protein content of plant withdrawal seeds and roots was measured at 595 nm. The Bradford method involved creating a stock solution of bovine serum albumin in a mass of 2 mg/ml for PBS and 1 mg/ml for Tris NaCl sample extracts. Three milliliters of Bradford reagent were combined with twelve milliliters of refined autoclaved water to create the reagent, which was then diluted out one to four times. Before being used, the reagent was immediately purified using Whatman No. 0.6 paper. The Bradford reagent was made using the specified recipe [19].

3. Results

3.1. The Percentage of Plant extract yield

The yield of the plants' extracts by ethanol method was between 2.50 and 5.50 g of dried plant material were left after 50 ml of ethanol. *Matricaria chamomilla* was produced the highest yields, followed by *Alhagi graecorum* extracts as shown in **Table 1**.

Table 1: The Percentage of Plant extract yield.

Plant Species	Family	Local Name	Plant Part Used	Extract Yield (%)
Matricaria chamomilla	Asteraceae	Chamomile	Flowers	9.85
Alhagi graecorum	Fabaceae	Manna tree	Roots	8.75

3.2. Minimum Inhibitory Concentrations (MICs)

The results demonstrated that, all plant extracts were successful in preventing the microbiological growth of bacteria that cause urinary tract infections at concentration 15 mg/ml, *Matricaria chamomilla* extract was the most effective against *S. aureus* and *P. aeroginosa* while *Alhagi graecorum* extract was the most effective against *E. coli*, and *S. pyogen*, as shown in **Table 2** and **Figure 1**.

Table 2: Antimicrobial activity of ethanol extract (15 mg/ml) against some bacteria.

Plant Species	Inhibition Zones (mm)			
	Gram Positive bacteria		Gram Negative bacteria	
	S. pyogen	*S. aureus*	*E. coli*	*P. aeruginosa*
Matricaria chamomilla	12.8	20.6	14.5	16.8
Alhagi graecorum	14.5	13.5	17.5	12.5

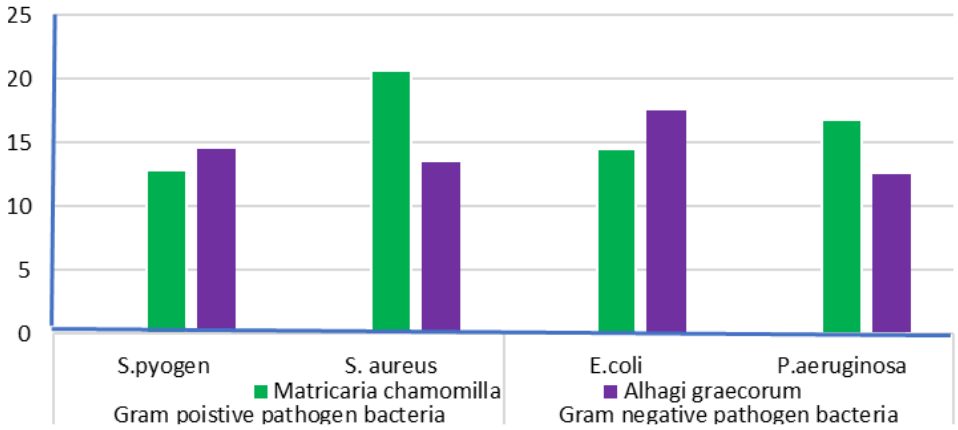

Figure 1: Antimicrobial activity test of ethanol plants extract (15 mg/ml) against some. pathogenic bacteria.

3.3. Minimum Bactericidal Concentrations (MBC)

With the exception of "*Pseudomonas aeruginosa*", which was less susceptible and had a" MIC of 13.5 mg/ml, *Matricaria chamomilla* extract demonstrated possible bactericidal activity against the tested pathogenic bacteria *S. aureus and E. coli* at a bactericidal concentration of 8 mg/ml, whereas *Alhagi graecorum* extract had a bactericidal concentration of 15 mg/ml.

Both *Matricaria chamomilla* and *Alhagi graecorum* may be used to prevent and control bacterial urinary tract infections, according to the MBC and MIC results of the active plant extracts. The significance of the bacterial strains in the "urinary tract" led to their inclusion in this investigation. *Matricaria chamomilla* extract demonstrated activity against three bacterial *Pseudomonas aeruginosa, streptococcus pyogenes and Escherichia coli*" strains but was less effective against *Staphylococcus aureus*. The plant extract, was found to inhibit the growth of all tested bacterial strains.

4. Discussion

In this study, the extract of ethanolic chamomile leaves showed antibacterial activity against the MDR *P. aeruginosa* isolates. Thus, it can be used in the production of antibacterial agents, and it is a good option for protection against pathogenic microorganisms, as well as *P. aeruginosa*.therefore The results demonstrated that, all plant extracts were successful in preventing the microbiological growth of bacteria that cause urinary tract infections at concentration 15 mg/ml, Matricaria chamomilla extract was the most effective against S. aureus and *P. aeroginosa* while Alhagi graecorum extract was the most effective against *E. coli*, and *S. pyogen.*Authors in [21] used concentrations of 50, 100, 200, and 400 µl/disc to report the antibacterial activity of *Ricinus communis* seed" against pathogens using Tris NaCl buffer". The ethyl acetate

extract of *Pseudowintera colorata* against *S. aureus* and the methanol extract of Kunzea ericoides against *Bacillus cereus* and *Candida albicans* had the lowest MICs, 62.5 µg/ml. Furthermore, when tested against *S. aureus and B. cereus* methanol extract and *S. aureus* ethyl acetate extract, K. ericoides also showed the lowest MLC of 250 µg/ml. *B. cereus* was killed by Weinmannia racemosa methanol extract (MLC = 250 µg/mL) [22]. In line with a work by [23]. the present investigation found no inhibitory effect of chamomile ethanol extract against isolates of *P. aeruginosa* using the agar well diffusion method . Although an Iraqi study showed different values 32 and 64 mg/ml, our study MIC and MBC were found to be 12.5 mg/ml and 15 mg/ml respectively [24]. The outcomes of [25] Four plant extracts *Peganum harmala*, *Piper nigrum*, *Syzygium aromaticum*, and *Cinnamomum zeylanicum* were examined for their antibacterial properties against Proteus mirabilis, which was isolated from urinary tract infections. For every type of extract, a concentration of 10 mg/ml was employed. For every type of extract, a concentration of 10 mg/ml was employed. With inhibition zones of 18 mm, 15 mm, and 14 mm, respectively, the aqueous extract demonstrated efficacy against *Proteus mirabilis* at this dose, but the extract of *Cinnamomum zeylanicum* displayed no inhibition zone. In accordance with [26], which documented the outcome of antibacterial susceptibility to *S. aromaticum*, our findings of clove *Syzygium aromaticum* demonstrated antibacterial activity against "Proteus mirabilis" with an inhibition zone of 14 mm. In aqueous extract, the average diameter zone of inhibition for *Proteus mirabilis, S. aureus, and P. aeruginosa* was "14.33 mm, 8 mm, and 31 mm, respectively [27], offered more evidence in favor of these conclusions.

5. Conclusion

Natural organic molecules serve as the active ingredients in plant extracts, which have a complex structure. They continue to be a useful tool in the battle against infections that are resistant to drugs in spite of these difficulties. To fully utilize the therapeutic potential of antimicrobial peptides in infection control, future research should concentrate on addressing these constraints.

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